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Note

Comparative susceptibilities of twelve insect cell lines to infection by three baculoviruses

Insect cell lines are commonly used to study insect viruses (Blissard, 1996) and have often been considered for production of certain virus species as biopesticides or for recombinant proteins. In the case of proteins, the *Spodoptera frugiperda* line IPLB-Sf21AE and the Sf-9 clone derived from it have been most frequently used with the baculovirus expression vector derived from *Autographa californica* nucleopolyhedrovirus (Ac-MNPV). However, many other lepidopteran cell lines are permissive to infection by this virus.

In the present study, twelve insect cell lines (Table 1) were tested for susceptibility to baculovirus infection by use of a typical endpoint assay procedure (Lynn, 2002) using extracellular virus (ecv) suspensions of three nucleopolyhedroviruses (NPVs), including *A. californica* NPV (AcMNPV), *Anagrapha falcifera* NPV (AfMNPV), and *Anticarsia gemmatalis* NPV (AgMNPV). Each of these viruses was originally isolated from a noctuid moth species and infect many species from this family as well as some other Lepidoptera. By using the same virus dilutions on each line and scoring for infection, the relative susceptibility of the cells can be determined for each virus. Cultures were maintained in 25 cm² Greiner Cellstar tissue culture flasks (Frickenhausen, Germany) at 26 °C on a weekly subculture in-

terval. The specific media used for maintenance was the one that provided the best growth for each line and was used in the assays since this provides the most consistent results (Lynn, 2000). Cell identity was confirmed by isozyme analysis.

The resulting titers obtained with the various cell/virus combinations are presented in Fig. 1. Because of its wide use in baculovirus research, the Sf21 cell line was used as a benchmark in Fig. 1 by dividing the $TCID_{50}/ml$ (titer) obtained on each line by the value obtained with this line for each virus. This method eliminates the differences in actual titers for each virus suspension, making comparisons between viruses easier.

Sensitivity to infection is one aspect of virus replication that can vary between cell lines. I have previously shown the TN-R² line was more than 100-fold more susceptible to AcMNPV than the more commonly used Sf21 cell line (Lynn, 1992a). This was confirmed in the present study. The TN-R² line was also most susceptible to AfMNPV which is not surprising since the AfMNPV virus has a great deal of genetic homology to AcMNPV (Harrison and Bonning, 1999). Ag286 from the velvetbean caterpillar also was considerably more susceptible to these viruses than Sf21 and was most susceptible to its homologous virus, AgMNPV, by 600-fold over Sf21.

Table 1 Cell lines and media used

Species	Designation	Passage level ^a	Medium ^b	Reference
Spodoptera frugiperda	IPLB-Sf21AE	Very high	Ex-Cell 400	Vaughn et al. (1977)
Anticarsia gemmatalis	UFL-Ag286	480	TC-100-II	Sieburth and Maruniak (1988)
Heliothis virescens	IPLB-HvE1a	250	TC-100-II	Lynn and Shapiro (1998)
H. virescens	IPLB-HvE6a	260	TC-100-II	Lynn and Shapiro (1998)
H. virescens	IPLB-HvE6s	290	TC-100-II	Lynn and Shapiro (1998)
H. virescens	IPLB-HvT1	400	TNM-FH	Lynn et al. (1988)
Lymantria dispar	IPLB-LdEIta	690	Ex-Cell 400	Lynn et al. (1988)
L. dispar	IPLB-LdEp	560	Ex-Cell 400	Lynn et al. (1988)
Plutella xylostella	IPLB-PxE2	660	TC-100-II	Lynn et al. (1988)
Trichoplusia ni	TN-368	Very high	TNM-FH	Hink (1970)
T. ni	IAL-TND1	1090	IPL-52B-P	Lynn et al. (1982)
T. ni	IPLB-TNR	840	TNM-FH	Rochford et al. (1984)

^a Passage levels indicate the number of subcultures of each line at the beginning of the experiment. All test were completed within 20 passages. The strains of TN-368 and SF21AE that were used were obtained from other researchers at unknown levels but are well over 1000 for each.

^b ExCell400 is a serum free medium from JRH Biosciences; TC-100-II is a modified formulation of TC-100 (Lynn, 1996); TNM-FH (Hink and Strauss, 1976); and IPL-52B-P (Lynn et al., 1988).

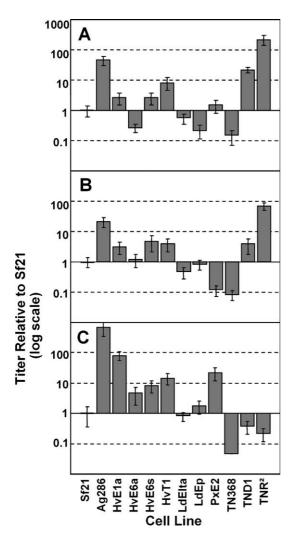


Fig. 1. Relative susceptibility of cell lines infected with nucleopolyhedroviruses. Virus inoculum of a clone of AcMNPV (AcMNPV-pxh) was as described previously (Lynn, 2002). Clones of AfMNPV (AfMNPV-sf) and AgMNPV (AgMNPV-ag3) were prepared by plaque purification on Sf21 and Ag286 cells, respectively. Titers were determined based on the presence of occlusion bodies in the nuclei after four weeks at 22 °C. Each bar is the titer of the cell line indicated on the *x*-axis divided by the titer for the same virus on Sf21 cells. TCID₅₀/ml and the standard errors (represented by error bars on the graph) were calculated by Spearman–Kärber (Lynn, 1992b). (A) AcMNPV, (B) AfMNPV, (C) AgMNPV.

This differs from the results obtained by Grasela and McIntosh (1998) with this same virus, since they showed greater sensitivity of their *Heliothis virescens* line as compared to their *A. gemmatalis* line. This elicits an interesting point found in these studies: the wide variability that can be seen between cell lines developed from the same species. The TN368 line (originally from adult ovaries) was substantially less susceptible to each virus while the TN-R² (from embryos) is substantially more susceptible to three viruses (Lynn, 1992a and the current results) and the susceptibility of the TND1 line (from imaginal discs) varied, being more susceptible to AcMNPV and AfMNPV but equal or less susceptible to

AgMNPV. These results indicate that differences in susceptibility of individual cell types exist in addition to species differences in susceptibility, possibly due to the tissue type involved. Goodman et al. (2001) also found substantial differences between cell lines from the same species in their studies with these same and some other viruses. Multiple cell lines from two other insect species (four from *H. virescens* and two from *Lymantria dispar*) were also examined in this study. For most of the viruses, the L. dispar lines showed results similar to Sf21 although LdEIta was substantially (8-fold) less susceptible to AcMNPV. More variability was seen with the various H. virescens lines. All four H. virescens lines were more susceptible to AgMNPV than was the Sf21 line, by 7- (HvE6a) to 90-fold (HvE1a). The HvT1 line from larval testes was 9- and 20-fold more susceptible to AcMNPV and AgMNPV, respectively. HvE1a and HvE6s were each 3- to 7-fold more susceptible to Ac-MNPV and AfMNPV while the HvE6a line was about an equal amount less susceptible to these viruses.

The three cell lines most distant from the original noctuid hosts of these viruses were the two gypsy moth lines (LdEIta and LdEp) and the diamondback moth cell line, PxE2. Like the gypsy moth lines, PxE2 had similar levels of infectivity to Sf-21 for AcMNPV. However, it was nearly 10-fold less susceptible to Af-MNPV and 20-fold more susceptible to AgMNPV. It will be interesting to determine if the larvae of this species have similar susceptibilities to these four viruses. However, the susceptibility of cell lines from a species is obviously not an absolute indicator of larval susceptibilities. The L. dispar lines are a case in point. While both the LdEIta and LdEp lines have an adequate level of susceptibility to AcMNPV, the larvae of this species have virtually no susceptibility to this virus per os (Dougherty, unpublished data). However, other cell lines from this species have been shown to be only semipermissive to this virus, resulting in no progeny virus (McClintock et al., 1986). This may suggest that by testing a sufficient variety of cell lines, one may be found which is predictive of larval infectivity. Ultimately, finding lines predictive of insect-susceptibility may also require use of occlusion-body derived virus, since it initiates infections in larvae.

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